ISOLATION OF BACILLUS COLI COMMUNIS FROM THE ALIMENTARY TRACT OF FISH AND THE SIGNIFICANCE THEREOF.*

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IN the selection of a public water supply it often happens that a decision regarding the sanitary character of the same is based almost wholly upon analytical evidence. For want of a better substitute, the colon bacillus has been quite generally accepted as an index to pollution; and although opinion varies widely regarding the absolute value of the colon test in sanitary water analysis, and although it is to be admitted that it has its limitations, yet nearly everyone, including the most doubtful, is inclined to look with suspicion upon a water, which, on analysis, discloses the presence of the colon bacillus.

More has been written in recent years regarding this organism and allied forms than upon almost any other topic relating to the bacterial analysis of water. The value of the qualitative test for B. coli communis has been questioned for three reasons; namely, (1) because the presence of a few of these organisms in a water does not necessarily mean that it is made unfit for primary purposes thereby; (2) because there are numerous saprophytic forms which show, upon study, cultural characteristics identical, or nearly identical, with those shown by the colon bacillus; and (3) because the fact that the colon bacillus is occasionally found in apparently pure waters does not necessarily imply that the typhoid bacillus is also present.

Of the more recent writers on the subject Prescott[1] has pointed out that bacteria corresponding in every way to B. coli are not confined to animal intestines, but are widely distributed elsewhere in nature. He says: "Certain lactic-acid bacteria isolated from sources apparently free from contamination are absolutely identical with the colon bacillus." Moore,[2] on the other hand, states

* Read at the meeting of the Laboratory Section of the American Public Health Association, October 26, 1903.

that, no matter if the colon bacillus is found elsewhere than in the digestive tract of living animals, including man, to his knowledge it has never been satisfactorily demonstrated in any substance where the possibility of tracing it to this source was precluded. The demonstration of this organism in sources other than the digestive tract, in sewage and in water, does not appear to the writer to depreciate the value of the colon test in water analysis as a perhaps presumptive, but valuable, index to fecal contamination.

Other cases than those cited above are constantly noted where opinion regarding the value of the colon test is divided. Be this as it may, it is still the case that, even in the face of adverse criticism, the presence of B. coli communis in water is widely considered as an unfavorable indication, and the sanitary character of such water suffers materially in consequence.

It is, of course, well known that the reason for such wide recognition of the value of this organism as an index to fecal pollution lies in the fact that it is essentially a germ of sewage origin. Some writers have even gone so far as to claim that B. coli communis and B. typhosus are one and the same, possessing divergent cultural characteristics at times, but under favorable conditions capable of exerting a similar action upon man in bringing about enteric disorders.

It is not the purpose of this paper to attempt to unravel the badly knotted skein of evidence for or against these theories. It is enough to assume that B. coli communis has its specific value in indicating the impurity of a water supply. It is enough to be assured that this organism and B. typhosus are found together, even though it is known that, while the former is always present where the latter is found, the latter is not always found to exist in common with the former. It is an indisputable fact that B. coli communis is a constant inhabitant of intestinal matter. It is equally certain that it is not a constant inhabitant of sources other than this.

It is the opinion of the writer that, if the value of the colon test in sanitary water analysis is depreciating, it is not because it lacks substantial ground as a basis for its claims

as an index to fecal contamination, but because laboratory methods for its study are not improving along systematic lines with the rapidity or certainty that are imperative. As Moore[2] has very aptly pointed out, a closer knowledge of the but indifferently understood forms connecting the accepted colon bacillus and its prototype is required. It is true that there are many forms of bacteria which closely resemble B. coli, but the fact they fail in some specific test does not prove, in the opinion of the writer, that they are not B. coli. It is equally true that the colon bacillus at times will reach a stage of deterioration where it will not respond to one-half the prescribed tests. But it is the belief of the writer, that if preliminary cultivation is resorted to before final results of growth are recorded, a much closer adherence to fixed characteristics will be noted in the study of the colon bacillus. A more thorough preliminary cultivation than was suggested some three years ago[3] might not be unwise, and it would appear that, by taking advantage of this point, bacteriologists may not only be able to bring together the numerous varieties of the colon bacillus under one head, but also always to obtain constant results in a study of this organism in water.

ISOLATION OF BACILLUS COLI COMMUNIS FROM FISH.

There appear to the writer but three ways in which the colon bacillus may become the inhabitant of a water; namely: through the introduction of sewage, through the direct agency of man and animals, and through the agency of fish.

Some time ago the writer arrived at the decision that fish might well be admirable vehicles for the transportation of bacterial life from one body of water to another. It appeared perfectly feasible to him that a fish in its passage from a polluted into a comparatively pure water might carry with it the colon bacillus which, if subsequently isolated therefrom, would entitle the water under study, in accordance with existing ideas, to a place among suspicious, even if not polluted, waters, and unfit for domestic consumption.

So far as is known to the writer, there is no published literature bearing directly on this subject, although I am informed by

Mr. G. C. Whipple that something in this line was done by him some years ago. In Mr. Whipple's study of the intestinal contents of some fifty or seventy-five fresh-water fish he was unable to find the colon bacillus. It is worthy of note, however, that the fish studied by him — including trout, white perch, and sunfish — were caught in an obviously unpolluted water.

Occasion offered itself for a study of the point in question at St. Louis, Mo., during the year of 1900, and the results of this work appear to the writer to be of sufficient interest to be placed on record. Acknowledgment here is due to the assistance of Mr. C. A. Snodgras, who in a painstaking manner worked out the results given below. The writer wishes also to express his indebtedness to Dr. Amand Ravold for many valuable suggestions during the course of the work.

In preparing for this work, arrangements were made whereby freshly caught fish of well-known species were expressed to the laboratory, and immediately upon their arrival were opened, and tubes of broth* inoculated with the contents of the large intestine and stomach of each.

From these cultures, after twenty-four hours' incubation at body temperature, lactose litmus agar plates were prepared, three plates to each tube. These plate cultures were incubated for from twenty-four to forty-eight hours at body temperature, and characteristic colonies transferred therefrom to fermentation-tubes containing dextrose broth. These cultures were allowed forty-eight hours' incubation at 37° C., and at the expiration of that time, if gas was absent from any of the tubes, further study in such cases was discontinued. Work in the case of the tubes showing gas production was continued as follows:

Lactose litmus agar plates were prepared, three plates from each fermentation-tube, and incubated for from twenty-four to forty-eight hours at body temperature. Characteristic colonies were selected from these plates, and usually two or three stock cultures were prepared on slant agar in the case of each plate. When the stock cultures had developed sufficiently, the following

*Liebig's beef extract, 10 g.; peptone, 10 g.; dextrose, 10 g.; Parietti solution, 20 c.c.; distilled water, 1,000 c.c. Reaction before addition of Parietti solution, neutral to phenolphthalein.

media were inoculated therefrom: nutrient broth tube, gelatin tube, fermentation tube, peptone solution for indol, nitrate solution, and litmus milk.

The cultures giving the following reactions were judged to be B. coli communis:

Bacillus, true form, obtained in three-day agar cultures.

Motility in two-day broth cultures.

Broth rendered turbid.

Gelatin tube, luxuriant growth along the entire path of the needle, without liquefaction of the gelatin.

Fermentation of dextrose broth with a total gas production of from 25 to 75 per cent. Ratio of H to CO_2 approximately 2 : 1.

RESULTS OF TESTS FOR BACILLUS COLI COMMUNIS IN THE ALIMENTARY TRACT OF FISH.

Fish	Where Caught	Number Examined	Number in Which B. Coli Communis was Found	
			Intestine	Stomach
German carp	Illinois River	13	9	8
German carp	Mississippi River	3	..	1
Buffalo	Illinois River	4	4	3
Buffalo	Mississippi River	5	5	3
Spoonbill cat	Mississippi River	1	1	..
Silver carp	Illinois River	2	1	..
Silver carp	Mississippi River	4	4	1
Gar	Illinois River	1	..	..
White perch	Illinois River	7	2	2
White perch	Mississippi River	4	2	1
Black bass	Illinois River	1	..	..
Cat	Illinois River	7	5	1
Cat	Mississippi River	2	2	1
Sunfish	Mississippi River	1	1	..
Eel	Illinois River	1	1	..
Eel	Mississippi River	1	..	..
Croppie	Mississippi River	1	..	..
Sturgeon	Illinois River	1	1	1
Sturgeon	Mississippi River	2	..	1
Tooth herring	Mississippi River	2	1	1
Sucker	Mississippi River	2	1	..
Dogfish	Illinois River	1	1	..
Dogfish	Mississippi River	1	..	..

Total number of fish examined - - - - - - - - - - 67
Total number of fish from which B. coli communis was isolated - - 47
Number of fish in the stomach of which B, coli communis was found - 24
Number of fish in the intestines of which B. coli was found - - - 41

Indol produced.

Nitrate reduced to nitrite.

Milk coagulated without liquefaction of the casein; litmus reddened.

Fecal odor of growth in broth and agar cultures, respectively.

All of the above tests, except where specifically stated, were made after ten days' incubation at body temperature. The results of these studies are given above.

MULTIPLICATION OF B. COLI COMMUNIS IN THE INTESTINAL TRACT OF FISH.

Further work was done to determine whether this organism multiplied to any extent in the intestinal tract. Infusions were prepared by suspending the intestinal contents of several fish in distilled water. These infusions, after sterilization, were inoculated with the colon bacillus. Having in mind the fact that cooked organic matter offers better opportunity for the nourishment of bacterial life than otherwise, infusions were also prepared by suspending the intestinal contents in sterilized distilled water and filtering the same through a Berkefeld filter, instead of resorting to sterilization as in the case of the other infusions. These latter infusions, while not found to be absolutely sterile in all cases, were practically so. They were also inoculated with the colon bacillus.

The results of this experiment showed a very pronounced increase in numbers of B. coli after three days' standing at a temperature of 20° C., not only in the infusions sterilized before inoculation, but in the filtered infusions as well. Original plates made from the inoculated infusions showed from 100 to 1,000 coli per cubic centimeter, while after three days the number had increased to many hundreds of thousands.

CONCLUSIONS.

It has been shown that the colon bacillus is taken up by fish, and the results of the experiment described above indicate that it multiplies rapidly in the intestinal tract of the same. It has been shown that a search of a large number of fish caught in an unpol-

luted water failed to reveal the presence of this organism in the intestinal tract. It would seem possible, therefore, that fish, having taken up the colon bacillus from a polluted water might migrate to a water of comparative purity, where they would naturally discharge the greatly increased number of these organisms.

Whether the finding of the colon bacillus deposited in a water in this manner would depreciate the value of the colon test in the examination of a public water supply is a question. It would certainly appear that, if the colon bacillus can be thus easily transferred from one water to another, the transportation of the typhoid bacillus may be considered quite as likely. At least the above results may explain the apparent phenomenon frequently noted where B. coli communis is found in comparatively large numbers in waters apparently open to but remote chances for fecal contamination.

REFERENCES.

1. PRESCOTT. *Amer. Pub. Health Assn. Repts.*, 1902, 28, pp. 413 ff.
2. MOORE. *Ibid.*, 1902, 28, pp. 397 ff.
3. MOORE. *Ibid.*, 1899, 25, pp. 580 ff.